THE OCCURRENCE OF THE SPORES OF B. BOTULINUS IN THE HAWAIIAN ISLANDS AND CHINA. VII *

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The predominance of B. botulinus, type A, in the Pacific Coast States is in striking contrast to that of type B in the Atlantic States, England and Europe. These differences in the distribution of types cannot be attributed to any topographic factors but coincide amazingly with the density of the population and agricultural activity. It naturally suggests itself to compare these observations with those found in specimens collected in other portions of the Western Hemisphere and in certain parts of the Eastern Hemisphere. The Hawaiian Islands have been chosen on account of their mountainous surface and their volcanic origin. The soil is very fertile, being formed by the disintegration of volcanic rocks and the decay of vegetable matter. These islands possess geologically a soil stratum which in all respects is very similar to that of the Pacific Coast and Sierra Nevada mountains. Furthermore, there is considerable agricultural activity in form of cane sugar and pineapple plantations.

The distribution of B. botulinus on the Asiatic continent has been studied on specimens obtained from China. This republic is heavily populated and is one of the most important agricultural countries of the Far East. Fertilization with sewage and animal manure has been practiced for centuries. The soil is known to contain B. tetani and other pathogenic anaerobes. A study of the soil specimens offers, therefore, data of comparative value.

EXPERIMENTAL DATA

Three series of specimens have been examined by the technic employed in similar studies. Guinea-pigs as well as mice have been used for the identification of toxic enrichment cultures.

Hawaiian Islands.—Through the courtesy of Mr. R. I. Bentley, President of the California Packing Corporation, 23 samples of soil and vegetables were procured from Wahiawa, of the Island of Oahu, in April, 1921. Cultures were made of the following specimens:

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Five soil samples.

Eighteen specimens of banana roots, pineapple roots with manure (4); grass, papaia roots, tomato plants, parsley, radish, turnip, beet, strawberries, papaia fruit, banana, mulberries, cabbage, tops and roots of strawberries.

One sample of pineapple root, papaia root and mulberries produced weakly toxic cultures.

In January, 1922, one of us (P. S.) collected and made cultures of 16 additional samples of soil and feeds from the Island of Oahu. The cultures gave the following results:

One soil—Algarobagrove—not cultivated, about 150 ft. from a stable, B. botulinus, type B.

One surface soil, 6 inches from surface (equine botulism outbreak?), Farm I., B. botulinus, type B.

One deep soil, 1 ft. down near stable, farm 2, B. botulinus, type B.

One surface soil, same place, B. botulinus, type B.

One virgin surface soil—Tantalus, about 6 inches deep, B. botulinus, type A.

One virgin soil side road, 2 ft. down "Round Top," B. botulinus, types A and B.

One soil, same place suface soil, B. botulinus, type B.

One good grade rice straw, weak toxin. Manure pile, drainage, 200 ft. from stable, rice straw on farm 1, garden soil on "Round Top," lava deposits, soil from Tantalus, wet and dry tarrow patch in Manoa Valley (8), all negative.

Summary: 17 soil cultivated or manured, 5 type B; 2 virgin soil in mountain region, 1 type A and 1 type A and B; 20 vegetables and feed, 4 weak toxins.

Total: 39 samples with 7 (11) or 17.9 (28.2) %, positive cultures.

China.—In May, 1922, the laboratory received from Dr. Carl Ten Broeck, Peking Union Medical College, China, 52 specimens of soil collected from the following provinces and towns:

Thirty-two Chilili: Peking, 18 (near grave, 3; cultivated fields, 9; ground covered with trees, 2; temple ground near Lo TzLung AnTom, 1; cleared ground, 3). Near Temple Heaven, 14 (cleared ground, 6; ground covered with trees, 8).

Twenty Shansi: Near town of Kao Chia Chuang, 12 (cultivated field, 1; ground covered with trees, 8; freshly cleared field never cultivated, 1; cleared ground, 1; open forest, 1). Near town of Tzi Lao, 4 (hillside forest, 1; clear ground covered with trees, 3). Near town of Chang Kou, 1 (cleared ground,1). Near town of Ping Ting Chow, 2 (cultivated field, 2). Near town of Le Ping, 1 (cultivated garden, 1).

The cultures gave the following results:

Three cultivated field (no decayed vegetable matter present), Peking, 3 type B.

One temple ground near the door of Lo Tz Lung An Tom, Peking, 1 type B.

One near grave, 1 weak toxin.

Three ground covered with trees, near town of Temple Heaven, Peking, 2 type B, 1 weak toxin.

Two cleared ground, near town of Temple Heaven, Peking, 1 type B, 1 weak toxin.

Three ground covered with trees, near town of Kao Chia Chuang, Shansi, 1 type B, 2 weak toxins.

Two cleared ground, no decayed vegetable matter present, near town of Chang Kou, Shansi, 1 type A and 1 type B.

One hillside forest (open), near town of Tzi Lao, 1 type B.

One freshly cleared field, some roots, etc., Kao Chia Chuang, Shansi, 1 type B.

Two ground covered with trees, near town of Tzi Lao, 2 type B.

One cultivated field garden near town of Le Ping, 1 weak toxin.

Summary: 12 cultivated fields with and without decayed vegetable matter, 3 type B, 1 B. tetani; 21 grounds covered with trees, some without decayed vegetation, 5 type B, 3 weak toxins, 1 type A; 11 cleared ground with and without decayed vegetation, 2 type B, 1 weak toxin; 3 near grave, 1 weak toxin; 1 hillside forest, abundant vegetation, 1 type B; 1 open forest on steep hill (large amount of decayed pine needles) ———; 1 cultivated garden, probably manure, 1 weak toxin; 1 temple ground, 1 type B (weak); 1 freshly cleared field, decayed vegetable matter present, 1 type B.

Total: 52 samples with 14 (20) or 26.9 (38.4) %, positive cultures.

Eleven, or 28.2%, of the 39 soil and vegetable samples collected in the Hawaiian Islands produced toxic enrichment cultures. The toxin of B. botulinus was identified in 7, or 17.9%, of the samples by antitoxin neutralization. Five cultures contained B. botulinus, type B. while one was neutralized by a type A and another by a polyvalent antitoxin. Four cultures prepared from fruits, roots and rice straw yielded weak toxins. The spores B. botulinus, type B, were quite frequently encountered in the soil of the Island of Oahu. Surface as well as deep soil, and virgin as well as cultivated soil, carried the organism. B. botulinus, type A, was found alone only in one virgin soil specimen, but in another instance was present with type B in a surface mountain soil. Type B is the principal toxicogenic anaerobe. This observation apparently confirms our opinion that type B exists primarily in fertile soils which are favored by the decay of vegetable matter and abundant rainfall. The percolating rain water is probably responsible for the presence of B. botulinus spores in the deeper layers of the soil. Conditions favorable for generation of toxins in moldy straw, etc., are unquestionably present, and it is therefore not surprising to learn that one outbreak of equine botulism has been observed on the premises of the U. S. Aviation Field on Oahu. The laboratory studies presented in this paper have demonstrated the presence of the anaerobe in the rice straw and the soil of the farm on which the outbreak occurred. Major James S. Simmons, M. C., isolated the B. botulinus from the spleen of one of the horses. It is most unfortunate that at the time of the outbreak the suspected hay was not studied for the determination of the presence of B. botulinus toxin. Human cases of botulism have thus far not been recognized in the Hawaiian Islands.

The observations made on the 52 soil samples procured from two coast provinces in China duplicate those of the Hawaiian Islands,

except that the percentage of toxic cultures is somewhat higher, namely, 38.4%. One enrichment culture prepared from a specimen of soil derived from cleared grounds near Kao Chia Chuang was highly toxic and contained B. botulinus, type A. B. botulinus, type B, obtained in all the other specimens, is geographically widely distributed, but judging from the toxicity of the cultures, it is quite evident that the soil contains relatively few spores. The majority of toxins were fatal to guinea-pigs in not less than 36 to 72 hours. Grounds covered with trees, some showing decayed vegetation, yielded the largest percentage of toxic cultures. A few soils obtained from forests, etc., harbored the same type, and it is impossible to offer any final explanations for the predominance of type B in the samples examined. The evidence strongly indicates that this type is indigenous to the lowland of China. A study of soils collected in the Khingan Mountains or the interior of China, which is less heavily populated than the provinces of Chilili and Shansi, should be made in order to amplify the results thus far obtained. Geographic and telluric studies on the distribution of the various types of B. botulinus tend to emphasize one fact—the soils of the New World, and particularly the Cordilleran mountain system of North America, harbor type A, while countries with old civilizations, as, for example, China and Europe and some of the Atlantic and Middle Western States of America, contain B. botulinus, type B., either exclusively, or at least predominantly. The surveys thus far completed unquestionably offer scattered and fragmentary evidence of a distinct process of bacterial mutation, and it is along these lines that the distribution of B. botulinus should be further investigated. The problem of microbial evolution or the adaptive development of bacterial species to their environment receives in the light of the geographic studies on B. botulinus a new impetus, and it is hoped that additional work on the presence of this anaerobe in South America, India, Africa and Australia may be undertaken in the not too distant future.

CONCLUSIONS

B. botulinus, type B, is frequently found in the soils obtained from the Island of Oahu, in the Territory of Hawaii, and from the provinces of Chilili and Shansi, in China. Type A has been found only in two Hawaiian and in one Chinese soil specimen.